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TITLE: Noninvasive Optical Monitoring of Spinal Cord Hemodynamics and Oxygenation after Acute Spinal Cord Injury

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14. ABSTRACT

Our objectives in Year 1 are fully addressed. Simultaneous to obtaining institutional (UBC) and ACURO approvals, a comprehensive assessment on current NIRS technology advancements related to tissue hemodynamics monitoring was performed. That study helped us to design and develop our first NIRS system (V1) required for monitoring spinal cord oxygenation and perfusion in our animal model of Experiment 1. We also identified available standard NIRS systems that were required to examine function and calibration of the V1 sensor. To monitor spinal cord cytochrome c redox state (CCR), we designed a NIRS system prototype (OXT5) that uses a novel multi-wavelength hardware and algorithm. Intellectual Property (IP) protection of our developed systems and algorithms were initially assessed through UBC University Industrial Liaison Office (UILO). We will follow up our UILO IP application after completion of Experiment 1. A pilot animal study with five pigs was conducted during the Y1-Q3 period to evaluate our NIRS sensors prototypes. The pilot study helped us to modify the protocol of Experiment 1 and refine some technical aspects of V1 and OXT5 NIRS sensors. We then started Experiment 1 as scheduled in Y1-Q4. Four animals are studied so far, and data collection and comparative analysis are under process. In year two we will complete Experiment 1 and focus on refining the technology to engineer a sensor that can be used in Experiment 2, a pig study with a seven-day post-injury survival period.

15. SUBJECT TERMS

Spinal Cord Injury, Hemodynamic Support, Spinal Cord hemodynamics, Spinal Cord Blood Flow, Near Infrared Spectroscopy, Intraparenchymal Pressure

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1 INTRODUCTION

The hemodynamic management of acute spinal cord injury (SCI) represents an under-appreciated opportunity to improve neurologic recovery in human patients. A major limitation in our ability to optimize hemodynamic management in acute SCI is the lack of a real-time method for measuring blood flow, oxygenation, metabolic responses, and hydrostatic pressure within the injured spinal cord. Near-infrared spectroscopy (NIRS) offers the potential to provide a relatively non-invasive measure of these important parameters within the injured spinal cord. NIRS works by transmitting near infrared light through tissue, and based on the absorption of this light by chromophores such as oxygenated and deoxygenated hemoglobin (O_2Hb and HHb), microcirculatory oxygen and perfusion can be derived. Additionally, alterations in the O_2Hb waveform that are caused by tissue pressure can be potentially utilized to monitor changes in hydrostatic pressure within the cord. Finally, NIRS measures of the redox state of cytochrome-c-oxidase (CCO) can provide information not just about tissue O_2 but also about downstream cellular O_2 metabolism. The overall objective of this initiative is to develop an implantable NIRS sensor and system that can be used to provide non-invasive real-time measurements of spinal cord oxygenation, blood flow, pressure, and oxidative metabolism in acute human SCI. We will test the hypothesis that a NIRS sensor positioned extra-durally can provide real-time measurements of tissue oxygenation, perfusion, oxidative metabolism and hydrostatic pressure within the underlying spinal cord adjacent to the site of traumatic injury over the course of seven-day post-injury. To test this hypothesis, we will conduct a series of preclinical studies using our pig model of thoracic SCI, alongside efforts to refine the NIRS technology into a clinically applicable device. Our animal studies aim to establish the relationship between non-invasive NIRS measurements of oxygenation, perfusion, metabolism and pressure with invasive IP monitoring that is made possible by the large calibre of the pig spinal cord. First, we will conduct a non-survival study in 8 animals to verify the relationship between NIRS and IP spinal cord monitoring after various intra-operative stimuli, including systemic hypoxia, contusive SCI, sustained spinal cord compression, and alterations in blood pressure. After refining the technology to engineer a sensor that can potentially be used in humans, we will test the NIRS system in another pig study with a seven-day post-injury survival period to determine how well the NIRS system monitors tissue changes in comparison to IP monitoring in awake, mobile animals (a more clinically relevant scenario).

2 KEYWORDS

- Spinal Cord Injury
- Hemodynamic Support
- Spinal Cord hemodynamics
- Spinal Cord Blood Flow
- Near Infrared Spectroscopy
- Intraparenchymal Pressure

3 ACCOMPLISHEMENTS

3.1 Protocol and Activity Status

- **Human Use Regulatory Protocols**
  No human subject research will be performed to complete the Statement of Work

- **Use of Human Cadavers for RDT&E, Education or Training**
  No RDT&E, education or training activities involving human cadavers will be performed to complete the Statement of Work

- **Animal Use Regulatory Protocols**

  Total Protocols: Two animal use research protocol will be required to complete the Statement of Work
- **Protocol: 1 of 1**
- **Protocol [ACURO Assigned Number]:** Conveyed from SC130007 and SC130008
- **Title:** Optical Monitoring of Spinal Cord Hemodynamics
- **Target required for statistical significance**: n=8 / each of two experiments
- **Target approved for statistical significance**: n=8 / each of two experiments
- **Submitted to and Approved by:** Bryan K. Ketzenberger, DVM, DACLAM
- **Status:** Approved - August 15, 2016

3.2 Approved Statement of Work

The approved statement of work is described below. A current Gantt chart is provided in Table 1 for reference.

Aim 1. Refine our current NIRS sensor (V1) configuration, and software algorithms to optimize the measurement of spinal cord (SC) oxygenation (O2Hb, HHb, TOI%), perfusion (THb), metabolism (CCO) and hydrostatic pressure.

Task 1: Submit documents for Institutional (UBC) and ACURO approval. [Months 1-3]
Task 2: NIRS technology assessment. [Months 1-6]
Task 3: Intellectual Property (IP) protection assessment, through UBC University Industrial Liaison office (UILO). [Months 1-6]
Task 4: Develop SC NIRS sensor V1 to be used in experiment 1. [Months 5-9]

Milestone(s) Achieved:
(a) *UBC and ACURO approvals are obtained.*
(b) *NIRS technology assessment is completed.*
(c) *The first round of the Invention Disclosure assessment is completed by the UBC University Industry Liaison Office (UILO).*

(d) *The first SC-NIRS sensor (V1) is developed. The first version of the V1 NIRS sensor prototype was designed, manufactured, modified and examined in a pilot study on five pig models of SCI during March 13 - May 8, 2017. The final version of the V1 NIRS sensor was prototyped through the middle of May and is now is being used in Experiment 1.*
e) *To address challenges associated with monitoring CCO, a sub-project was established to design and develop a novel custom-made NIRS sensor, different from the V1 NIRS sensor. Our developed CCO NIRS sensor was successfully operated and initially examined.*
(f) *The protocol of Experiment 1 was tested and refined.*

Aim 2. Verify the relationship between the NIRS and intraparenchymal (IP) assessments of oxygenation, perfusion, pressure, and metabolic responses in our pig SCI model in anesthetized (immobile) animals.

Task 1: Evaluating the NIRS system function in a pig model of SCI in conjunction with IP measurements of oxygen, perfusion, pressure, and metabolism. (Non-survival experiment with NIRS & Intraparenchymal monitoring under anesthesia, n=8)

Task 2: Comparing tissue oxygenation & perfusion as assessed by 1) NIRS measures of O_2Hb, HHb, THb, and TOI%, and 2) an intraparenchymal oxygen/blood flow sensor. [Months 10-14]

Task 3: Comparing hydrostatic pressure as assessed by 1) a novel analysis of the NIRS O_2Hb waveform that accounts for how cardiac-induced oscillations in the waveform are influenced by tissue pressure, and 2) an intraparenchymal pressure sensor. [Months 10-14]

Task 4: Comparing metabolic responses as assessed by 1) NIRS measures of cytochrome-c-oxidase (CCO), and 2) an intraparenchymal microdialysis catheter to monitor lactate, pyruvate, and the lactate/pyruvate ratio. [Months 10-14]

Task 5: Data analysis. [Month 15]

Milestone(s) Achieved:
(a) *Experiment 1 is started. We are collecting spinal cord NIRS data, using the V1 and OXT5 NIRS systems, in conjunction with IP measurements of spinal cord tissue oxygenation, perfusion, pressure, and metabolism in the first animal experiment. It is a non-survival experiment with NIRS & Intraparenchymal monitoring under anesthesia. Four animals have been studied by the end of the Q4 period.*
(b) *V1 and OXT5 NIRS systems were successfully evaluated.*
(C) *A new upgraded waterproof OXT5 NIRS sensor is developed.*
(d) *NIRS data, including O_2Hb, HHb, THb, as well as CC-Redox trace, were successfully collected from the spinal cord tissue at T9 level adjunct to impact zone (T8) in four animal subjects of Experiment 1. Data were collected at baseline, pre-SCI hypoxia events, SCI and compression, decompression, post-SCI hypoxia events, MAP increase, MAP decrease and post-euthanasia. Initial qualitative analysis of collected data indicates a significant relationship between invasive IP and noninvasive NIRS measures of spinal cord tissue oxygenation and perfusion in the majority of physiological events of the protocol in four animal subjects of Experiment 1. Final data analysis will be performed upon completion of Experiment 1 in Year 2-Q1-Q2.*

Aim 3. Refine and miniaturize the NIRS sensor, cables, and fixation devices to develop an implantable (but also removable), NIRS sensor (V2) that can be used to monitor the

spinal cord for a clinically relevant time frame post-injury in the "non-ideal" setting of an awake (and moving) individual.

Task 1: NIRS monitoring system adjustment/refinement for long term (7-day) continuous monitoring, data storage and management. [Months 16-20]
Task 2: Sensor prototyping and development. [Months 16-19]
Task 3: Sensor calibration and testing. [Months 19-20]

Milestone(s) Achieved:
Nothing to report

Aim 4. Establish the ability of this refined NIRS system to assess oxygenation, perfusion, pressure, and metabolic responses for 7-day post-injury by comparing the NIRS and IP measurements in pigs that are awake and mobile in their cages for 7 days post-injury.

Task 1: Comparing changes in tissue oxygenation & perfusion as assessed by 1) NIRS measures of O_2Hb, HHb, THb, and TOI%, and 2) an intraparenchymal oxygen/blood flow sensor. [Months 21-28]
Task 2: Comparing changes in hydrostatic pressure as assessed by 1) a novel analysis of the NIRS O_2Hb waveform that accounts for how cardiac-induced oscillations in the waveform are influenced by tissue pressure, and 2) an intraparenchymal pressure sensor. [Months 21-28]
Task 3: Comparing changes in metabolic responses as assessed by 1) NIRS measures of cytochrome-c-oxidase (CCO), and 2) an intraparenchymal microdialysis catheter to monitor lactate, pyruvate, and the lactate/pyruvate ratio. [Months 21-28]
Task 4: Data analysis and dissemination. [Month 29]

Milestone(s) Achieved:
Nothing to report

Aim 5. Obtain local ethics approval to conduct a clinical trial of our finalized NIRS system in human acute SCI patients.

Task 1: Final refinements and development of a clinical sensor (V3) for a future pilot trial in human. [Months 30-34]
Task 2: Obtaining Canada Health Approval for the V3 sensor. [Months 32-36]
Task 3: Obtaining UBC CREB approval for conducting a clinical trial in acute SCI patient to test the V3 feasibility and safety. [Months 32-36]

Milestone(s) Achieved:
Nothing to report

Table 1: Approved Statement of Work (Gantt chart).

Specific Aim 1: *Refine our current NIRS sensor (V1) configuration, and software algorithms to optimize the measurement of spinal cord oxygenation (O_2Hb, HHb, TOI%), perfusion (THb), metabolism (CCO) and hydrostatic pressure.*	**YEAR 1**				**YEAR 2**				**YEAR 3**			
Activity	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
1a) UBC & ACURO approvals												
1b) Technology assessment												
1c) IP assessment												
1d) V1 Sensor development												
Specific Aim 2: *NIRS system evaluation and verification of the relationship between the NIRS and IP assessments of oxygenation, perfusion, pressure, and metabolic responses in our pig SCI model in anesthetized (n=8 immobile) animals (Experiment 1)*	**YEAR 1**				**YEAR 2**				**YEAR 3**			
Activity	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
2a) NIRS system evaluation and collecting NIRS and IP data in Experiment 1												
2b) Data analysis: NIRS vs. IP measurements (SC oxygenation & perfusion)												
2c) Data analysis: NIRS vs. IP measurements (SC pressure)												
2d) Data analysis: NIRS vs. IP measurements (SC metabolic responses)												
2e) Final data analysis and dissemination												
Specific Aim 3: *Refine and miniaturize the NIRS sensor to develop an implantable (but also removable), NIRS sensor*	**YEAR 1**				**YEAR 2**				**YEAR 3**			

(V2) that can be used to monitor the spinal cord for a clinically relevant time frame post-injury in the "non-ideal" setting of an awake (and moving) individual.												
Activity	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
3a) V2 sensor development												
3b) V2 sensor calibration												
3c) NIRS system refinement for long term (7-day) monitoring												
Specific Aim 4: *Establish the ability of V2 sensor to assess oxygenation, perfusion, pressure, and metabolic responses for 7-day post-injury by comparing the NIRS and IP measurements in pigs that are awake and mobile for 7 days post-injury. (n=8)*	**YEAR 1**				**YEAR 2**				**YEAR 3**			
Activity	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
4a) NIRS vs. IP measurements (SC oxygenation & perfusion)												
4b) NIRS vs. IP measurements (SC pressure)												
4c) NIRS vs. IP measurements (SC metabolic responses)												
4d) Data analysis, dissemination												
Specific Aim 5: *Obtain local ethics approval to conduct a clinical trial of our finalized NIRS system in human acute SCI patients.*	**YEAR 1**				**YEAR 2**				**YEAR 3**			
Activity	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
5a) Final refinements of V3 sensor												
5b) Obtaining Canada Health approval for the V3 sensor												
5c) Obtaining UBC CREB approval												

3.3 Current Progress on Statement of Work

A Gantt chart of the current work is provided in Table 2 for reference. The months in this Gantt chart reflects actual completed works.

Aim 1. Refine our current NIRS sensor (V1) configuration, and software algorithms to optimize the measurement of spinal cord (SC) oxygenation (O2Hb, HHb, TOI%), perfusion (THb), metabolism (CCO) and hydrostatic pressure.

- **Task 1:** Submit documents for Institutional (UBC) and ACURO approval.

  Completed. *We have obtained UBC and ACURO approval. This protocol was approved by the University of British Columbia, Vancouver IACUC on January 31, 2016. Dated August 15, 2016, ACURO approval was conveyed to project protocol from USAMRMC protocols SC130007 and SC130008 which were previously approved for the use of swine.*

- **Task 2:** NIRS technology assessment.

  Completed. *An updated literature review on the technology applied in this project is completed. The NIRS technologies and engineering components required for the development of the first (V1) sensor prototype is defined. The first series of equipment related to V1 sensor development and Experiment 1 are provided.*

  The potential value of a newly described "spinal cord perfusion index," developed by our biophotonics consulting group, to monitor changes in spinal cord perfusion is under process.

- **Task 3:** Intellectual Property (IP) protection assessment, through UBC University Industrial Liaison office (UILO).

  In Progress. *Our intellectual property application is reviewed by the UBC UILO (file number of 17-037). We are requested to provide some further technical information and define a number of the system specifications including the CCO measurements. We are planning to provide the required information after completing our experiment 1.*

- **Task 4:** Develop SC-NIRS sensor V1 to be used in Experiment 1.

  Completed.

Aim 2. Verify the relationship between the NIRS and intraparenchymal (IP) assessments of oxygenation, perfusion, pressure, and metabolic responses in our pig SCI model in anesthetized (immobile) animals.

- **Task 1:** Evaluating the NIRS system function in a pig model of SCI in conjunction with IP measurements of oxygen, perfusion, pressure, and metabolism.

In Progress. *Initial evaluation of NIRS systems is completed. Experiment 1 (spinal cord NIRS data collection in conjunction with spinal cord IP measurements of oxygenation, perfusion, pressure, and metabolism) is started. Four animals are studied so far.*

- **Task 2:** Comparing tissue oxygenation & perfusion as assessed by 1) NIRS measures of O_2Hb, HHb, THb, and TOI%, and 2) an intraparenchymal oxygen/blood flow sensor.

  In Progress. *Data collection and comparisons are in progress.*

- **Task 3:** Comparing hydrostatic pressure as assessed by 1) a novel analysis of the NIRS O_2Hb waveform that accounts for how cardiac-induced oscillations in the waveform are influenced by tissue pressure, and 2) an intraparenchymal pressure sensor.

  In Progress. *Data collection and comparisons are in progress.*

- **Task 4:** Comparing metabolic responses as assessed by 1) NIRS measures of cytochrome-c-oxidase (CCO), and 2) an intraparenchymal microdialysis catheter to monitor lactate, pyruvate, and the lactate/pyruvate ratio.

  In Progress. *Data collection and comparisons are in progress.*

- **Task 5:** Final data analysis and dissemination.

  Nothing to report

Aim 3. Refine and miniaturize the NIRS sensor, cables, and fixation devices to develop an implantable (but also removable), NIRS sensor (V2) that can be used to monitor the spinal cord for a clinically relevant time frame post-injury in the "non-ideal" setting of an awake (and moving) individual.

- **Task 1:** NIRS system adjustment/refinement for long term (7-day) continuous monitoring of the spinal cord, data storage and management.

  Nothing to report

- **Task 2:** Sensor prototyping and development.

  Nothing to report

- **Task 3:** Sensor calibration and testing.

  Nothing to report

Aim 4. Establish the ability of this refined NIRS system to assess oxygenation, perfusion, pressure, and metabolic responses for 7-day post-injury by comparing the

NIRS and IP measurements in pigs that are awake and mobile in their cages for 7 days post-injury.

- **Task 1:** Comparing changes in tissue oxygenation & perfusion as assessed by 1) NIRS measures of O_2Hb, HHb, THb, and TOI%, and 2) an intraparenchymal oxygen/blood flow sensor.

  Nothing to report

- **Task 2:** Comparing changes in hydrostatic pressure as assessed by 1) a novel analysis of the NIRS O_2Hb waveform that accounts for how cardiac-induced oscillations in the waveform are influenced by tissue pressure, and 2) an intraparenchymal pressure sensor.

  Nothing to report

- **Task 3:** Comparing changes in metabolic responses as assessed by 1) NIRS measures of cytochrome-c-oxidase (CCO), and 2) an intraparenchymal microdialysis catheter to monitor lactate, pyruvate, and the lactate/pyruvate ratio.

  Nothing to report

- ***Task 4:*** *Data analysis and dissemination.*

  Nothing to report

Aim 5. Obtain local ethics approval to conduct a clinical trial of our finalized NIRS system in human acute SCI patients.

- **Task 1:** Final refinements to develop a clinical sensor (V3) for a future pilot trial in human.

  Nothing to report

- **Task 2:** Obtaining Canada Health Approval for the V3 sensor.

  Nothing to report

- **Task 3:** Obtaining UBC CREB approval for conducting a clinical trial in acute SCI patient to test the V3 feasibility and safety.

  Nothing to report

Table 2: Gantt chart of current work. The Gantt chart reflects actual work completed. This Gantt chart of current work matches the Gantt chart of approved statement of work (Table 1).

Specific Aim 1: *Refine our current NIRS sensor (V1) configuration, and software algorithms to optimize the measurement of spinal cord oxygenation (O_2Hb, HHb, TOI%), perfusion (THb), metabolism (CCO) and hydrostatic pressure.*	**YEAR 1**				**YEAR 2**				**YEAR 3**			
Activity	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
1a) UBC & ACURO approvals												
1b) Technology assessment												
1c) IP assessment												
1d) V1 Sensor development												
Specific Aim 2: *NIRS system evaluation and verification of the relationship between the NIRS and IP assessments of oxygenation, perfusion, pressure, and metabolic responses in our pig SCI model in anesthetized (n=8 immobile) animals (Experiment 1)*	**YEAR 1**				**YEAR 2**				**YEAR 3**			
Activity	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
2a) NIRS system evaluation and collecting NIRS and IP data in Experiment 1												
2b) Data analysis: NIRS vs. IP measurements (SC oxygenation & perfusion)												
2c) Data analysis: NIRS vs. IP measurements (SC pressure)												
2d) Data analysis: NIRS vs. IP measurements (SC metabolic responses)												
2e) Final data analysis and dissemination												
Specific Aim 3: *Refine and miniaturize the NIRS*	**YEAR 1**				**YEAR 2**				**YEAR 3**			

sensor to develop an implantable (but also removable), NIRS sensor (V2) that can be used to monitor the spinal cord for a clinically relevant time frame post-injury in the "non-ideal" setting of an awake (and moving) individual.												
Activity	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
3a) V2 sensor development												
3b) V2 sensor calibration												
3c) NIRS system refinement for long term (7-day) monitoring												
Specific Aim 4: *Establish the ability of V2 sensor to assess oxygenation, perfusion, pressure, and metabolic responses for 7-day post-injury by comparing the NIRS and IP measurements in pigs that are awake and mobile for 7 days post-injury. (n=8)*	**YEAR 1**				**YEAR 2**				**YEAR 3**			
Activity	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
4a) NIRS vs. IP measurements (SC oxygenation & perfusion)												
4b) NIRS vs. IP measurements (SC pressure)												
4c) NIRS vs. IP measurements (SC metabolic responses)												
4d) Data analysis, dissemination												
Specific Aim 5: *Obtain local ethics approval to conduct a clinical trial of our finalized NIRS system in human acute SCI patients.*	**YEAR 1**				**YEAR 2**				**YEAR 3**			
Activity	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
5a) Final refinements of V3 sensor												
5b) Obtaining Canada Health approval for the V3 sensor												
5c) Obtaining UBC CREB approval												

4 OVERALL PROJECT SUMMARY

The overall objective of this project is to develop a near-infrared spectroscopy (NIRS) system that can provide real-time monitoring of spinal cord oxygenation, blood flow, pressure, and metabolic responses after acute spinal cord injury (SCI). The ability to monitor these parameters within the injured spinal cord will provide clinicians with potentially critical information to optimize the hemodynamic management of the acutely injured patient. In this grant, we propose a sequence of preclinical studies aimed to translate this approach to human SCI patients.

4.1 METHODS

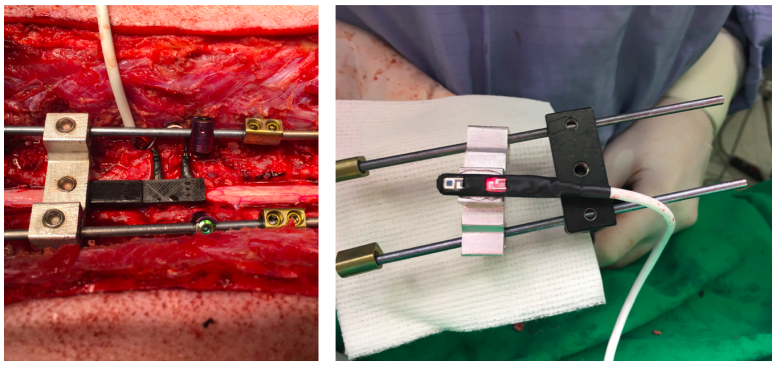
NIRS Technology Assessmsnet

An updated literature review and NIRS technology assessment related to spinal cord tissue hemodynamics monitoring was performed. That study helped us to design and develop our first NIRS prototype (V1) required for monitoring spinal cord oxygenation and perfusion in our animal model of Experiment 1. We also identified available standard NIRS systems that were required to examine function and calibration of the V1 sensor. Following the technology assessment and after extensive consultations with various NIRS manufacturers, such as Hamamatsu, we came to the conclusion that currently available NIRS technology and equipment do not have the capacity to reliably and accurately monitor spinal cord tissue cytochrome c oxidase (COO) activity, at least in a manner that would be applicable to eventual clinical application. Almost all researchers conducting CCO measurements are using experimental lab-based systems that are not applicable in clinical settings. To approach this challenge, we worked with a biophotonics consulting company in January 2017 to design and prototype a unique NIRS system that may enable us to detect spinal cord CCO changes. Pathonix Innovation Inc. is a Canadian biophotonics technology consulting company that has been active in the field of design and customizing NIRS systems applied by the clinical researcher in different universities and institutions including US National Institute of Health (NIH).

NIRS Prototype Development

1- V1 NIRS - The design and fabrication of the V1 SC-NIRS sensor were completed in the Y1-Q2 period. This sensor and the NIRS system were examined during the pilot study (Y1-Q3). Following some technology refinements, the final V1 NIRS sensor is being used during Experiment 1 to monitor changes of spinal cord O_2Hb, HHb, and THb during physiological events of the protocol (Figure 1).

Figure 1 – V1 NIRS sensor placed over the spinal cord at T11-T12.



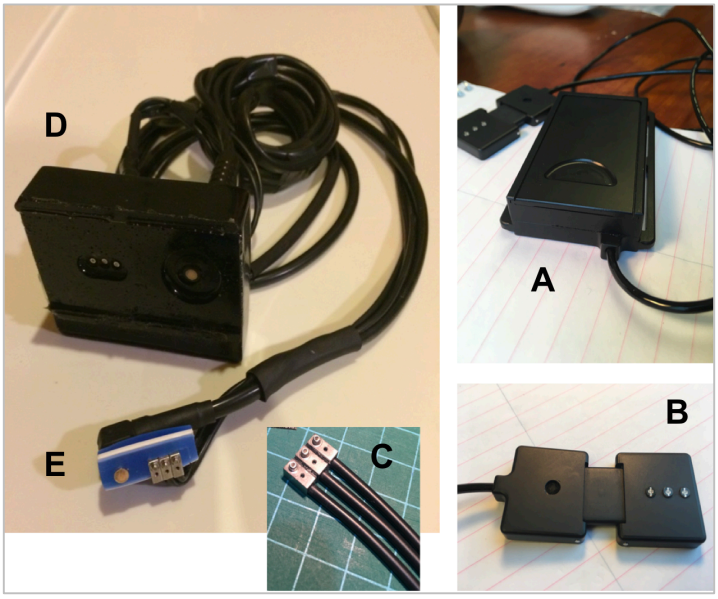
2- OXT5 NIRS – After consultations and reviewing the task with Pathonix engineers, a plan for developing a multi-wavelength NIRS prototype for monitoring changes of tissue CCO activity was proposed. A copy of the system development proposal is attached (Appendix 1). Upon accepting the proposal, the first CCO collector NIRS prototype was delivered on March 14, 2017. Further development and examination of the prototype required animal studies, which was arranged and completed during March-May. Following a series of sensor and software refinements, our team could collect three series of CC related traces using the OxiTor 5-wavelenght (OXT5) NIRS prototype (Figure 2). The first developed OXT5 prototype was not completely waterproof, which resulted in some malfunctions when there were bleeding around the sensor. To overcome this problem, in our request, Pathonix Inc upgraded OXT5 sensor to a waterproof model (Figure 3).

Figure 2 – OXT5 unit and the miniatorized sensor.

Figure 3 – Upgraded OXT5 sensor covered by an additional layer of clear silicon.

3- Porta-Light NIRS – To evaluate Optical NIRS set up, we made an optical sensor small enough to be placed over the spinal cord at T6 level. Using an optical converter the prototyped sensor was connected to a commercially available Spatially Resolved (SR) NIRS system (Figure 4). Using this sensor we also monitored spinal cord oxygenation and perfusion at T6 level.

Figure 4 – A) Porta-Light NIRS unit; B) Porta-Light NIRS sensor, size: 60 x 40 mm; C) optical fibers used in the converter unit; D) Converter unit that exchange NIRS light wavelengths between NIRS optodes and the customized small spinal cord sensor, size: 30 x 12 (E).



4- Sensmart NIRS – We used an additional NIRS system, an FDA approved commercial multi-channel NIRS device, to monitor regional oxygen saturation (rSO_2) of

the spinal cord as well as three control sites, the brain, the bladder and the thigh muscle (Figure 5). To monitor the spinal cord, we modified one sensor of the system to be placed over the spinal cord at the caudal T15 level (Figure 5C). Specifications of NIRS sensors are listed in Table 3.

Figure 5 – Sensmart NIRS system; A) Multi0-channel monitor; B) Standard NIRS sensor with 25 mm inter-optode distance; C) Modified NIRS sensor for placement over the spinal cord; D) One sensor placed over the animal's brain as a control side.

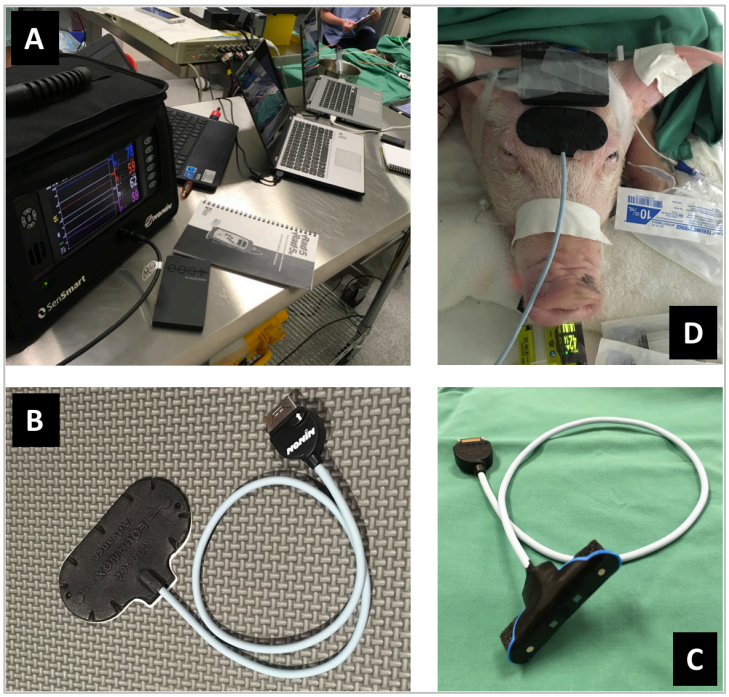


Table 3 – NIRS sensors that are placed over the spinal cord

	V1 NIRS	OXT5	PL NIRS	SS NIRS
Manufacturer	Pathonix	Pathonix	Artinis	Nonin Medical
Configuration	CW*	Multi-Wavelenght	Spatially Resolved	Spatially Resolved
Wavelenghts (nm)	670, 860	670, 770, 810, 850, 950	750, 860	730, 760, 810, 870
IOD (mm)**	12	10	20	25
Size (mm)	8 x 18	10 x 20	12 x 30	12 x 40
Measures	O_2Hb, HHb, THb,	O_2Hb, HHb, THb, TOI	O_2Hb, HHb, THb,	rSO_2

* CW – Continuous Wave, ** IOD – Inter-Optode Distance

Pilot Study

To examine the ability of our OXT5 NIRS system and sensor to detect traces of spinal cord CCR, we conducted a pilot study which involved monitoring of the spinal cord with the OXT5 sensor during a couple of hypoxic events and after euthanasia in an animal model of SCI. We believe that using this innovative NIRS system we successfully collected changes of CC Redox traces (CCR) within the spinal cord during hemodynamics manipulations. Beside standard NIRS monitoring, we continue collecting CCR traces during Experiment 1. A comprehensive analysis of CCR trace activity during physiological events of Experiment 1 will be performed in Y2-Q1.

Experiment 1

We use the porcine model of SCI as developed in our lab involving a combination of contusion and compression components. Oxygenation and hemodynamics of the spinal cord are also altered by inducing ventilatory hypoxia and changing mean arterial pressure (MAP) by infusing Noradrenaline and Nitroprusside.

OXT5 NIRS sensor monitors changes in spinal cord tissue O_2Hb, HHb, THb and TOI% at T9. V1 NIRS sensor monitors spinal cord tissue O_2Hb, HHb, and THb at T11. Signals collected by this sensor are used for TPSA analysis. Sensmart NIRS sensor monitors spinal cord rSO_2 at T15, and Porta-Light NIRS sensor monitors spinal cord tissue O_2Hb, HHb, and THb at T6.

Using Intraparenchymal (IP) catheters, spinal cord blood flow (SCBF), partial oxygen saturation (PaO_2), spinal cord hydrostatic pressure (SCP) and microdialysis of the spinal cord at 25 mm caudal to the center of the impact are interrogated during the experiment. Microdialyses samples are analyzed for various markers of cellular damage, ischemia and energy status, including lactate, pyruvate, L/P ratio and glucose.

Continuous monitoring of intraparenchymal spinal cord O_2 tensions (PaO_2) and blood flow is performed using the Oxylite system from Oxford Optronics. This probe consisted of a 4-channel composite containing a Laser Doppler Flowmetry probe with separate emitting and receiving channels, a fluorescent PO_2 probe, and a thermocouple. PaO_2 is defined as the partial pressure of oxygen in tissue and reflects the availability of oxygen for oxidative energy production. PaO_2 represents the balance between oxygen delivery and oxygen consumption. The oxygen portion of the Oxylite probe emits short pulses of blue LED light resulting in a fluorescent discharge that is quenched by tissue O_2. The signal is received by the Oxylite system and the O_2 tension using a factory-precalibrated algorithm expresses PaO_2 in mmHg. A thermocouple is included in the probe to correct for temperature.

Laser Doppler flowmetry, for measuring relative changes in perfusion, is based on the principle of the Doppler shift: Blood cells traversing tissue are struck by the light and reflect it, whereby the light undergoes a Doppler shift. The surrounding tissue also reflects the light but in an unshifted manner. Thus the volume of illumination is a mixture of an unshifted and a Doppler shifted component, the magnitude and frequency (wavelength) of the latter being related to the number of moving cells and their velocity. Microvascular blood perfusion therefore, is the product of mean blood cell velocity and

mean blood cell number concentration present in the small measuring volume of tissue under illumination from the probe.

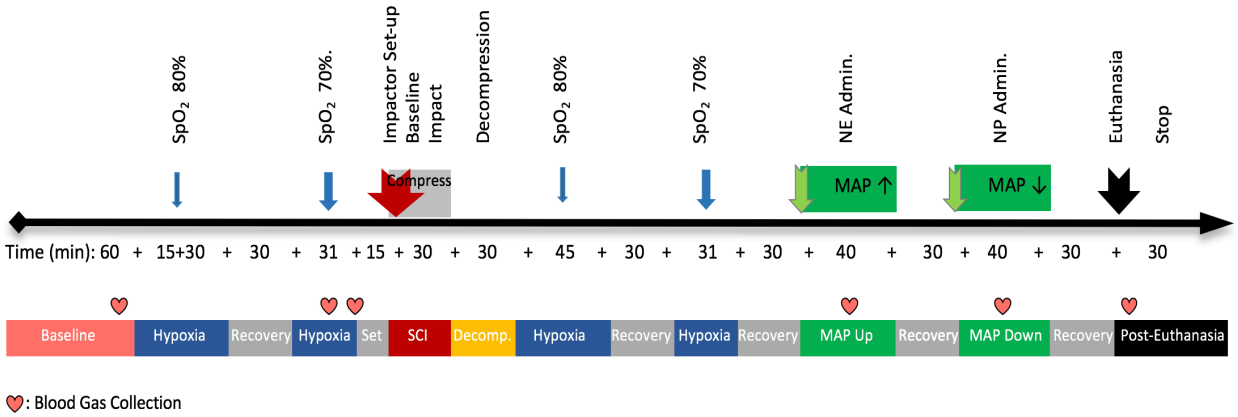
The technology of the intraparenchymal pressure sensor (FISO company) is based on the Fabry–Perot optical resonator at the end of the fibre, where one of the resonator walls is built as a membrane deflecting under pressure. Pressure changes inflict different wavelengths being amplified in the reflected spectrum. The conditioner that introduces light into the measuring fibre sensor is additionally equipped with a Charge-Coupled Device matrix for reflected light measurements, and because the relation between membrane deflection and pressure is linear, the pressure is measured by determining the membrane deflection.

The pilot study provided a valuable opportunity to review the protocol of Experiment 1. As a result of the pilot study, we came to this conclusion that applying some minor changes will improve the protocol of Experiment 1. To induce mild hypoxia pre- and post-SCI, we gradually increase the ventilatory ratio of Nitrogen/O_2 until SpO_2 drops to 80%. This method provides a more consistent episode of mild hypoxia. To induce severe hypoxia pre- and post-SCI, we stop ventilation until SpO_2 drops to 70%. Different studies have shown that pulse oximeter reading less than 70% (SpO_2) are not always reliable. Blood gas (BG) samples are collected from arterial and venous lines during 1) pre-SCI baseline measure, 2) pre-SCI severe hypoxia, 3) recovery period after first severe hypoxia, 4) MAP increase, 5) MAP decrease, and 6) two minutes' post-euthanasia. These values will be used for CC activity verification and calibration of the NIRS data. A fixed 30-minute recovery time is applied after each event of the protocol. This helps to have a better analysis of IP and NIRS data between physiological events.

Figure 6 shows the finalized protocol of Experiment 1. General timeline of Experiment 1 are as follow:

- 06:00am Animal placement and anesthesia
- 07:00am Urinary catheter placement
- 07:45am Femoral catheters placement
- 09:00am Jugular & carotid catheters placement
- 10:00am Laminectomy surgery
- 12:30pm Spinal cord ultrasound
- 12:45pm IP and NIRS sensors placement
- 02:00pm Data stabilization start
- 03:00pm Anesthesia stabilization
- 03:30pm Baseline measure
- 05:00pm Protocol starts
- 11:00pm Euthanasia
- 11:30pm Experiment end

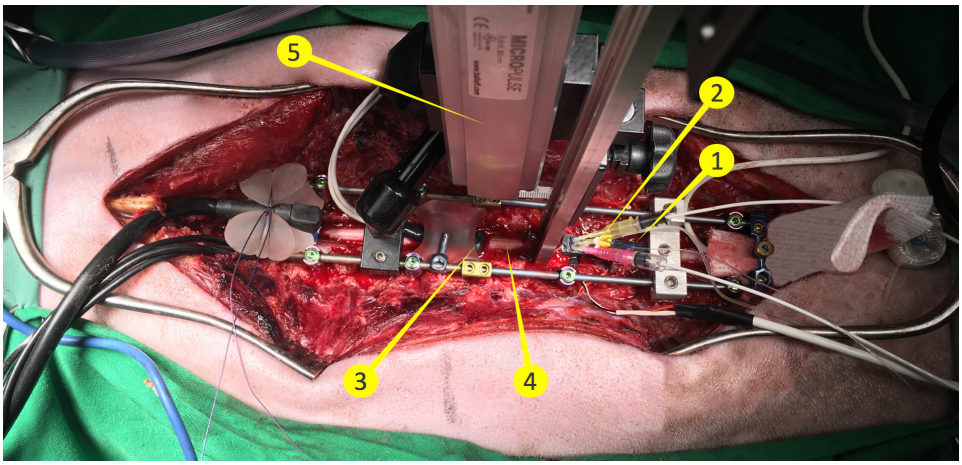
Figure 6- Expeiment 1 protocol-



Sensor Placement

Figure 7 demonstrates placement of NIRS sensors on the surgically exposed spinal cord along with invasive IP catheters inserted within the spinal cord.

Figure 7 – Sensor placements in Experiment 1 includes: 1) V1 NIRS sensor is placed at T12 level; 2) Intraparenchymal (IP) catheters are inserted at T11 level; 3) OXT5 NIRS sensor is placed on the spinal cord at T9 level; 4) Impact zone at T10 level; 5) The impactor device. Porta-Light NIRS sensor is placed at T6 and Sensmart NIRS sensor is placed at T15 level.

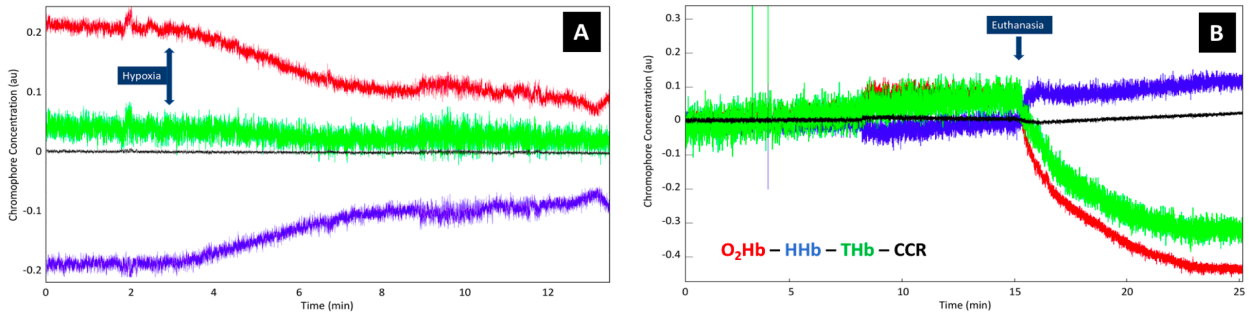


4.2 RESULTS

Pilot Study: As CC oxidation activity reflects the state of tissue subcellular energetics, as opposed to oxygen availability, a functional CC NIRS sensor should theoretically collect significant changes of CC Oxy/Redox (O/R) activity during cytopathic states. It is while significant changes of tissue CC-O/R activity during short periods of tissue

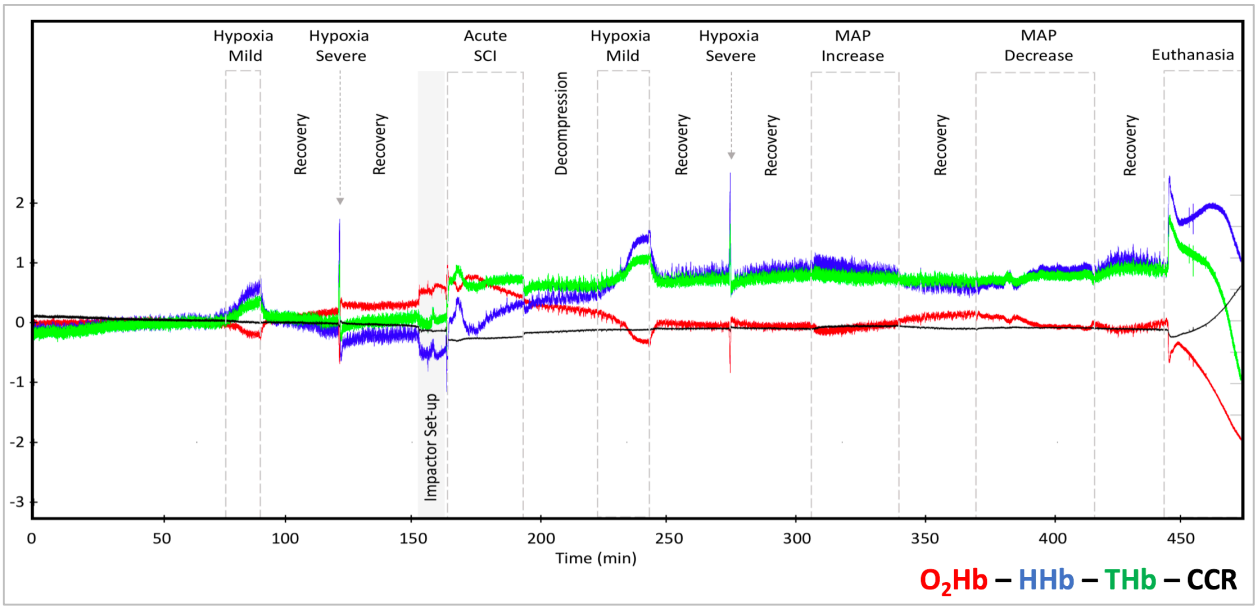
hypoxia is not expected as opposed to O_2Hb changes. Figure 8 demonstrates NIRS traces of the spinal cord in an animal pilot study pre- and post-euthanasia. This data, which is collected by OXT5 NIRS sensor, shows CC related traces with an independent pattern that was not following the O_2Hb trend. Subtle changes of this trace during a short term of hypoxia (Figure 8A), while changes of O_2Hb was significant, is indicative of CCR activity. The behavior of this trace after cellular death is also indicative of CCR activity (Figure 8B).

Figure 8- Changes of spinal cord O_2Hb, HHb, THb and CCR chromophores in an animal model of SCI during short term hypoxia (A) and post-euthanasia (B).



Experiment 1: NIRS data, including O_2Hb, HHb, THb, and CC-Redox trace, are successfully collected by OXT5 and V1 NIRS sensors in four animals of Experiment 1. Figure 9 represents one of the studies of Experiment 1. Data are collected at baseline, pre-SCI mild (SpO_2 80%) and severe (SpO_2 70%) hypoxia episodes, SCI and compression, decompression, post-SCI hypoxia episodes, MAP increase, MAP decrease and post-euthanasia, by OXT5 NIRS sensor.

Figure 9 – Overall NIRS data collected by OXT5 sensor at T9 during Experiment 1.



Upon induction of hypoxia, at both pre- and post-SCI, decreasing O_2Hb and increasing HHb show classic pattern of tissue hypoxia. Increase in THb during hypoxia episodes indicates tissue compensatory reaction to hypoxia by increasing heart rate and tissue vasodilation.

As CCO activity reflects the state of tissue subcellular energetics, CCR does not show significant changes during short hypoxia episodes of the protocol. Following impact (SCI) and compression, O_2Hb decrease and HHb increase indicate an overall SC tissue deoxygenation. This pattern, captured by NIRS, demonstrates the injurious effect of SC impact and compression. SC decompression reduces the magnitude of tissue deoxygenation by reducing the slope of HHb increase and O_2Hb decrease during 30 minutes of the protocol. Increasing MAP for 30 minutes improves SC tissue oxygenation as indicated by decreasing HHb and increasing O_2Hb levels. Decreasing MAP, however, impairs SC tissue oxygenation as indicated by HHb increase and O_2Hb decrease. Following euthanasia, an immediate sharp decrease of O_2Hb and increase of HHb indicate severe tissue hypoxia due to cardiorespiratory arrest. The overall decrease in THb, O_2Hb, and HHb during 30 min following euthanasia confirm tissue death. Interestingly, progressive rise in CCR trace confirms subcellular tissue death following euthanasia. The pattern of changes of all NIRS measures during different physiological events of Experiment 1 are consistent with what we would expect in response to induced hypoxia and spinal cord injury.

Figure 10 demonstrates a significant direct relationship between spinal cord oxygen partial pressure (PaO_2) changes measured by invasive Intraparenchymal (IP) catheter and changes of chromophore concentrations of O_2Hb as measured by non-invasive OXT5 NIRS sensor. Changes of SC PaO_2 following SCI (~140 – 270 min) and after euthanasia were not detectable. It is while O_2Hb could detect changes of SC oxygenation during all physiological events of the protocol. TOI% calculated by OXT5 NIRS sensor shows SC tissue oxygenation changes during all physiological events of the experiment (Figure 11).

There is a significant direct relationship between TOI% and PaO_2 measure. While changes in O_2Hb chromophore concentration provides relative changes in tissue oxygenation, TOI% measure provides an absolute measure of tissue oxygenation level, which is a significant advantage of this index in clinical NIRS studies.

Figure 12 shows the relationship between IP SCBF and NIRS THb data. In general, there is a direct relationship between IP SCBF and NIRS THb during this experiment.

Figure 10 – Relationship between IP PaO_2 and NIRS O_2Hb data during the entire protocol of Experiment 1 in one study at T9 level.

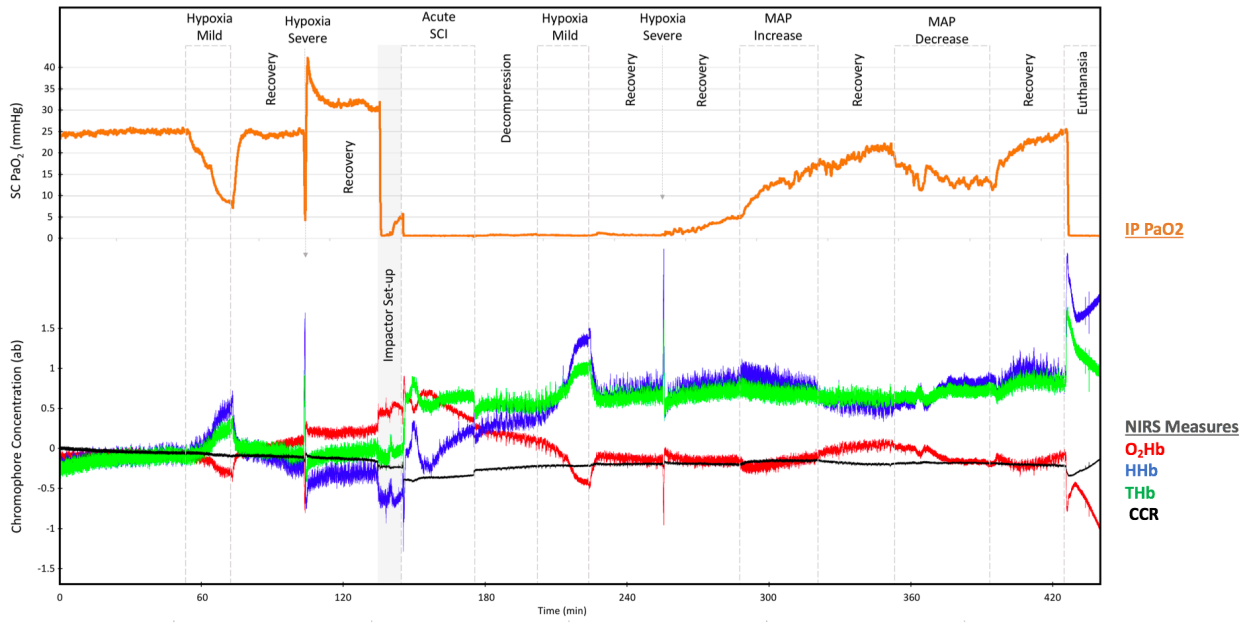


Figure 11 – Relationship between IP PaO_2 and NIRS-based TOI% during the entire protocol of Experiment 1 in the same study at T9.

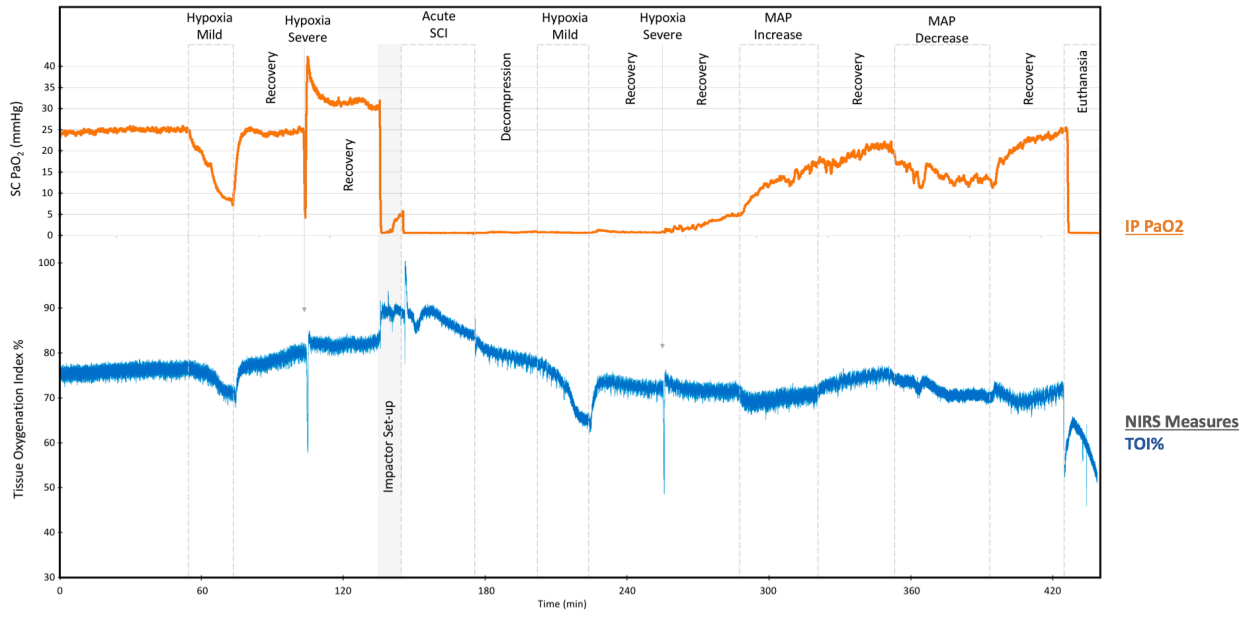
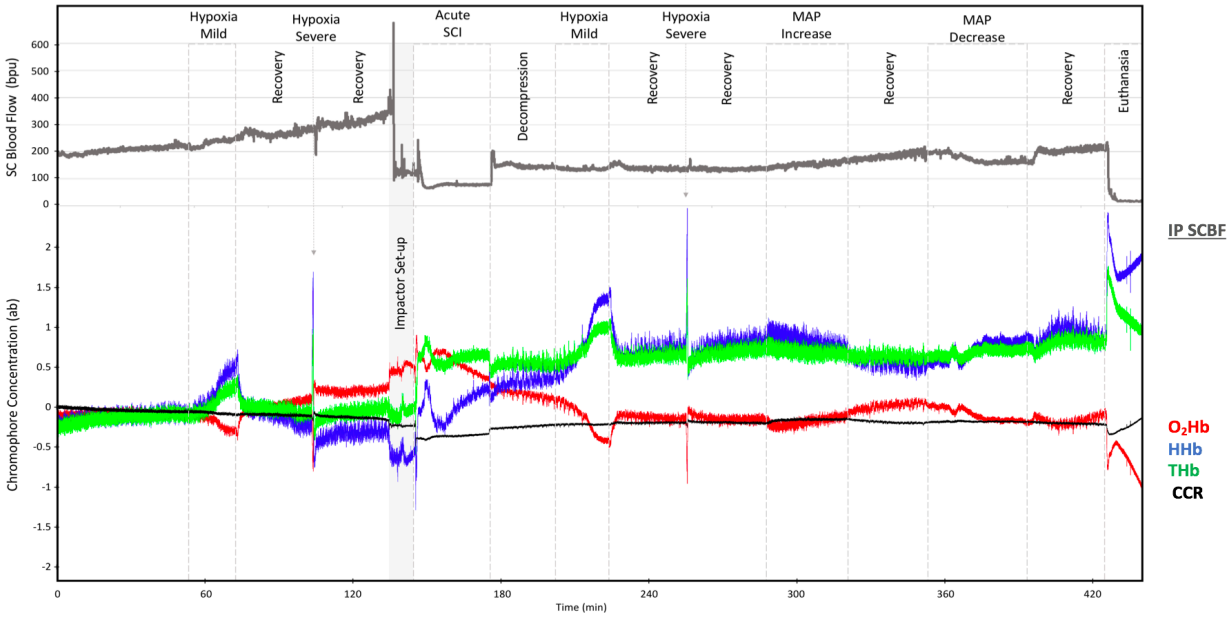


Figure 12 – Relationship between IP SCBF and NIRS-based THb during the entire protocol of Experiment 1 in the same study at T9.



Tissue Pulsation Signal Analysis (TPSA) is a non-invasive NIRS-based signal processing method, developed by our consultant company Pathonix, to evaluate hydrostatic tissue pressure by analysis of the effect of cardiac pulsation and respiration within the tissue of interest. This method is based on the processing of the signal characters of tissue arterial and respiratory pulsations. This method could detect lower limb compartments that were affected by compartment syndrome in previous studies. We aimed to evaluate the potential application of TPSA method to identify SC hydrostatic pressure, noninvasively, during physiological events of Experiment 1.

Figure 13 shows the relationship between IP spinal cord pressure (SCP) and TPSA data in one study of Experiment 1. Initial examination of TPSA method shows promises to identify changes in SC hydrostatic pressure. Further signal processing advancements are required to improve the method for monitoring SC tissue hydrostatic pressure.

With respect to spinal cord metabolism status as measured by microdialysis and spinal cord tissue CCR, a direct correlation between changes of Lactate to pyruvate (L/P) ratio and changes of CCR is evident (Figure 14).

L/P ratio is a marker for tissue ischemia, which increases significantly within minutes after SCI and cord compression and subsequently decreased after decompression. The direct relationship between L/P and CCR changes may, therefore, indicate CCR raise as a marker of tissue oxydative damage. This observation warrants further investigations.

Figure 13 – Relationship between IP SCP and TPSA data during the entire protocol of Experiment 1 in the same study at T9.

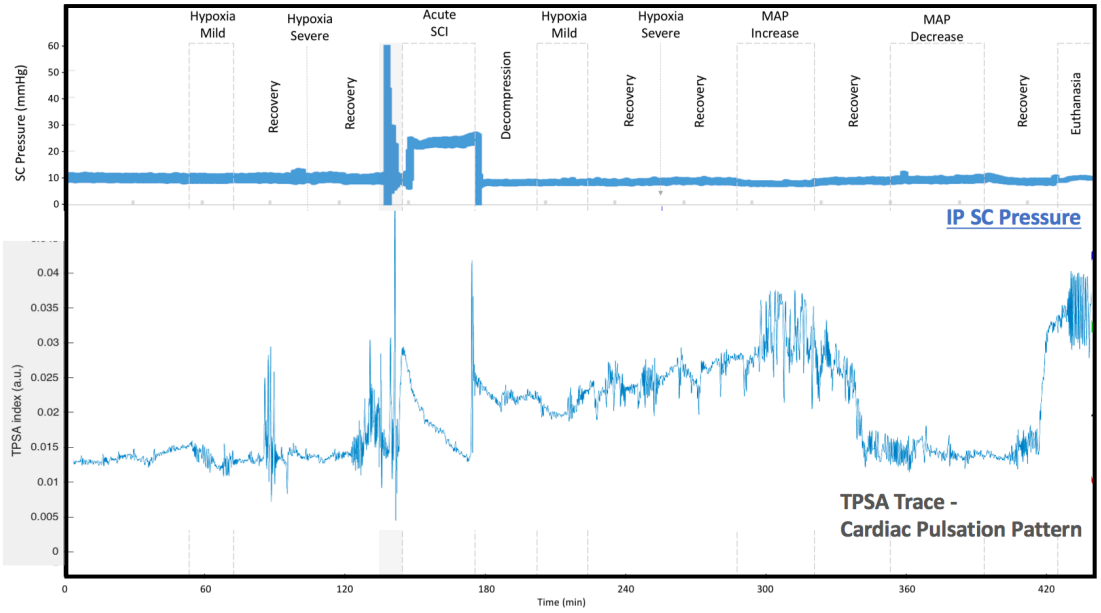
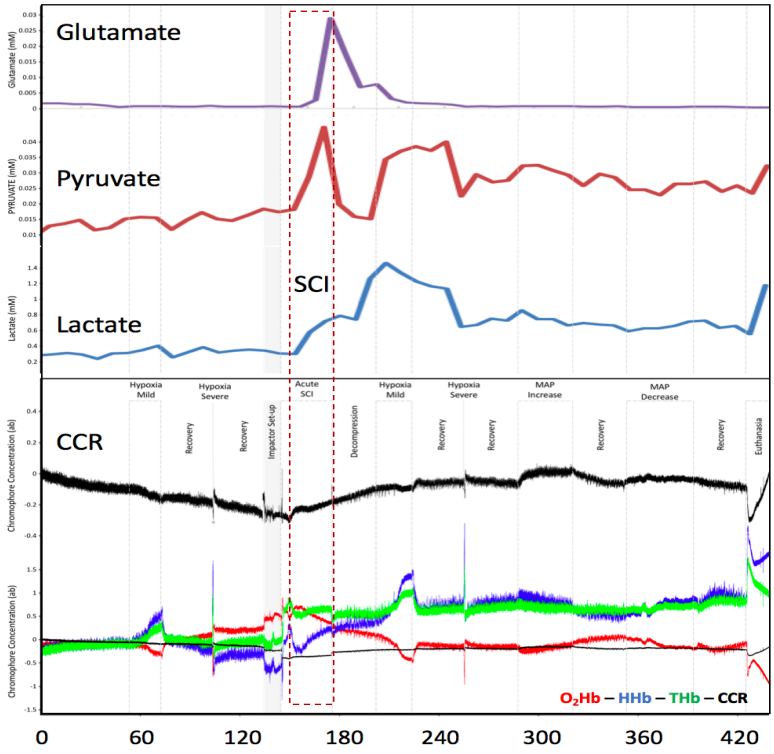


Figure 14 – Relationship between IP microdialysis Lactate, Pyruvate and Glutamate concentration changes and NIRS CCR changes during the entire protocol of Experiment 1 at T9 level.



In summary, we have performed four studies of Experiment 1. We will continue with the rest of the animals and will finish these surgeries by the end of 2017.

5 KEY RESEARCH ACCOMPLISHEMENTS

- V1 and OXT5 NIRS sensors and systems were successfully developed and tested.
- Research protocol of Experiment 1 was tested and refined during a pilot study.
- Experiment 1 is started as scheduled and four animals are studied so far.
- OXT5 NIRS sensor is collecting a novel trace that seems to be Cytochrome C Redox state of the spinal cord.
- OXT5 and V1 NIRS sensors were able to successfully collect validated pattern of tissue hypoxia, from the spinal cord, immediately after induction of ventilatory hypoxia.
- From collected data so far, consistent relationships between NIRS and IP measures are observed.
- NIRS TPSA method could identify major changes in hydrostatic pressure within the spinal cord following impact and decompression.

6 CONCLUSIONS

This research focuses on establishing a novel NIRS-based system and sensor that can be used to monitor spinal cord hemodynamics in the early post-injury period. Such an intervention will enable clinicians to optimize patient care by providing them real-time information about the physiology of the injured spinal cord. We have been successful to achieve our first year goals; to develop our novel spinal cord NIRS sensors and collect data from the spinal cord in an animal model of spinal cord injury. Upon successful completion and data analysis of Experiment 1, we will start the second round of NIRS technology development for continuous monitoring of spinal cord in Year 2.

7 PUBLICATIONS, ABSTRACTS, PRESENTATIONS

Podium Presentations:

1. "Optical monitoring of spinal cord hemodynamics, a feasibility study ", Optical Diagnostics and Sensing Conference, SPIE Photonics West Congress, San Francisco, USA, Feb 2017.

   Presented opn February 02, 2017.

2. "Optical monitoring of spinal cord subcellular damage after acute spinal cord injury" Babak Shadgan, Neda Manouchehri, Kitty So, Katelyn Shortt, Femke Streijger, Andrew Macnab, Brian Kwon. Paper Number: 10501-20.

   Accepted for Podium Presentation *at Optical Diagnostics and Sensing Conference, SPIE Photonics West Congress, San Francisco, USA, January 30, 2018.*

3. "In vivo near infrared (NIRS) sensor attachment using fibrin bioadhesive" Roberto Pagano, Andrew Macnab, Guy Dumont, Brian Kwon, Babak Shadgan. Paper Number: 10501-21.

   Accepted for Podium Presentation *at Optical Diagnostics and Sensing Conference, SPIE Photonics West Congress, San Francisco, USA, January 30, 2018.*

Optical Monitoring of Spinal Cord Hemodynamics

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Introduction: Spinal cord (SC) ischemia and hypoxia are important contributors to secondary damage after traumatic spinal cord injury (SCI). To mitigate these secondary processes and improve neurologic outcome, current clinical practice guidelines recommend aggressive maintenance of spinal cord perfusion and oxygenation. Such hemodynamic management, however, is currently carried out without any real-time measures of spinal cord perfusion and oxygenation. Such information would be extremely valuable to clinicians managing such patients in the intensive care setting. This study examined the feasibility and sensitivity of a custom-made near infrared spectroscopy (NIRS) sensor to monitor SC hemodynamics and oxygenation in a pig model of spinal cord injury.

Methods: Three anesthetized Yucatan mini-pigs were studied using a NIRS system with a miniaturized optical sensor applied directly on the surgically exposed SC at T9 during a set of systemic physiological manipulations including ventilatory hypoxia and altering mean arterial pressure (MAP). Three intraparenchymal probes were inserted through the dura at T11 to invasively monitor SC oxygenation, blood flow and pressure.

Results: Non-invasive NIRS monitoring reflected changes in intraparenchymal SC oxygenation and hemodynamics in response to ventilatory-induced hypoxia and changes in MAP. The changes in intraparenchymal oxygen level and blood flow were simultaneously reflected in the changes in NIRS oxygenated and deoxygenated hemoglobin concentrations.

Conclusions: This pilot study indicates that a novel miniaturized NIRS sensor has the potential to monitor SC hemodynamics and oxygenation in real time. Further development of this method may offer new options for improved SCI care.

Optical Monitoring of Spinal Cord Subcellular Damage After Acute Spinal Cord Injury

Babak Shadgan[1,2], Neda Manouchehri[2], Kitty So[2], Katelyn Shortt[2], Femke Streijger[2], Andrew Macnab[3], Brian K. Kwon[1,2]

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Introduction: Sudden physical trauma to the spinal cord results in acute spinal cord injury (SCI), leading to spinal cord (SC) tissue destruction, acute inflammation, increased SC intraparenchymal pressure, and tissue ischemia, hypoxia, and cellular necrosis. The ability to monitor SC tissue viability at subcellular level, using a real-time noninvasive method, would be extremely valuable to clinicians for estimating acute SCI damage, and adjusting and monitoring treatment in the intensive care setting. This study examined the feasibility and sensitivity of a custom-made near infrared spectroscopy (NIRS) sensor to monitor the oxidation state of SC mitochondrial cytochrome aa3 (Cytox), which reflects the subcellular damage of SC tissue in an animal model of SCI.

Methods: Six anesthetized mini-pigs were studied using a custom-made multi-wavelength NIRS system with a miniaturized optical sensor applied directly on the surgically exposed SC at T9. The oxidation states of SC tissue hemoglobin and Cytox were monitored before, during and after acute SCI.

Results: Non-invasive NIRS monitoring reflected changes in SC tissue Cytox redox state, simultaneous but independent of changes in hemoglobin saturation following acute SCI. A consistent increase in SC tissue Cytox redox state (0.046 [0.027 to 0.066] AU) was observed following SCI, indicating progressive SC cellular damage at the injury site.

Conclusions: This pilot study indicates that a novel miniaturized multi-wave NIRS sensor has the potential to monitor post-SCI changes of SC Cytox oxygenation state in real time. Further development of this method may offer new options for improved SCI care.

In vivo near infrared (NIRS) sensor attachment using fibrin bioadhesive.

Roberto Pagano[1], Andrew Macnab[2], Guy Dumont[1], Brian K. Kwon[3,4] Babak Shadgan[3,4]

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Background. Application of miniaturized near infrared spectroscopy (NIRS) sensors to monitor trends of hemodynbamics and oxygenation of an internal organ requires a temporary, stable and safe fixation. Bioadhesives are natural polymeric materials used as tissue adhesives and sealants for hemostasis and wound management. Such bioadhesives could potentially be utilized to secure implanted optical sensors. We evaluated 'Tisseel' (Baxter Healthcare, Deerfield, IL) a fibrin-based adhesive/sealant commonly used during spine surgery to augment dural repairs; investigating first if this bioadhesive adversely attenuates NIR photon transmission.

Methods. Photon transmission was investigated in both an in vitro and in vivo paradigm. For in vitro testing we used a 1-mm pathlength cuvette containing either air or 'Tisseel' interposed between a NIR light source (760, 850 nm) and a photodiode detector and compared transmittance. For in vivo testing a continuous wave (760, 850 nm) spatially-resolved NIRS device was placed over the triceps muscle using either conventional skin apposition (overlying adhesive bandage) or bioadhesion with 'Tisseel'. Raw optical data, chromophore concentrations and tissue saturation index values (TSI%) collected at rest were compared.

Results. In-vitro NIR light absorption by 'Tisseel' was very high, with transmittance reduced by 95% compared to air. In-vivo muscle TSI% values were 80% with conventional attachment and 20% using the bioadhesive.

Conclusion. While the nature and adhesive properties of 'Tisseel' suit NIRS sensor attachment in vivo this adhesive's optical properties critically compromise photon transmission. Other bioadhesives warrant being explored recognizing their unique properties and the growing need for indwelling sensor attachment in vivo.

8 INVENTIONS, PATENTS AND LICENSES

To explore the potential intellectual property that might be resulted from this project we approached the UBC University-Industry Liaison Office (UILO) and have filed an Invention Disclosure & Assignment application (file number of 17-037) in the Q1 period. Our invention disclosure was assessed by the UILO. The assessment included prior art searchers using the Thomson Innovation database as well as PubMed and Google Scholar for relevant publications. The prior art search identified a number of NIRS-based methods for monitoring spinal cord. However, none of them were similar to our design and capability of integrated real-time monitoring of spinal cord hemodynamics, oxygenation, and Intraparenchymal pressure. The UILO has determined that our system design is patentable. We are planning to revisit our potential IPs upon completion of Experiment 1 in 2018.

9 REPORTABLE OUTCOMES

Nothing to report

10 OTHER ACHIEVEMENTS

Nothing to report

11 REFERENCES

None

12 APPENDICES

Appendix 1 – Proposal for Design and Development of a Custom-made NIRS System.

APPEDIX 1

Proposal for Design and Development of a Custom-made NIRS System

Prepared for Dr. Brian Kwon
By Pathonix Innovation Inc.
December 29, 2016

SUMMARY

This proposal is for design and validation of a method for measuring CCO changes using an optical spectroscopic device along with the design and prototyping of a two-channel custom made NIRS system to implement the method. The proposal has been prepared following the meeting with Dr. Brian Kwon (the "Client") discussing the requirements for CCO measurement in the spinal cord injury investigation project. The device will measure tissue ΔO_2Hb, ΔHHb and potentially ΔCCO. The project involves design and prototyping the device and its operating software, supporting its tests in an animal study and providing expertise in analyzing and validating the results.

REQUIREMENTS

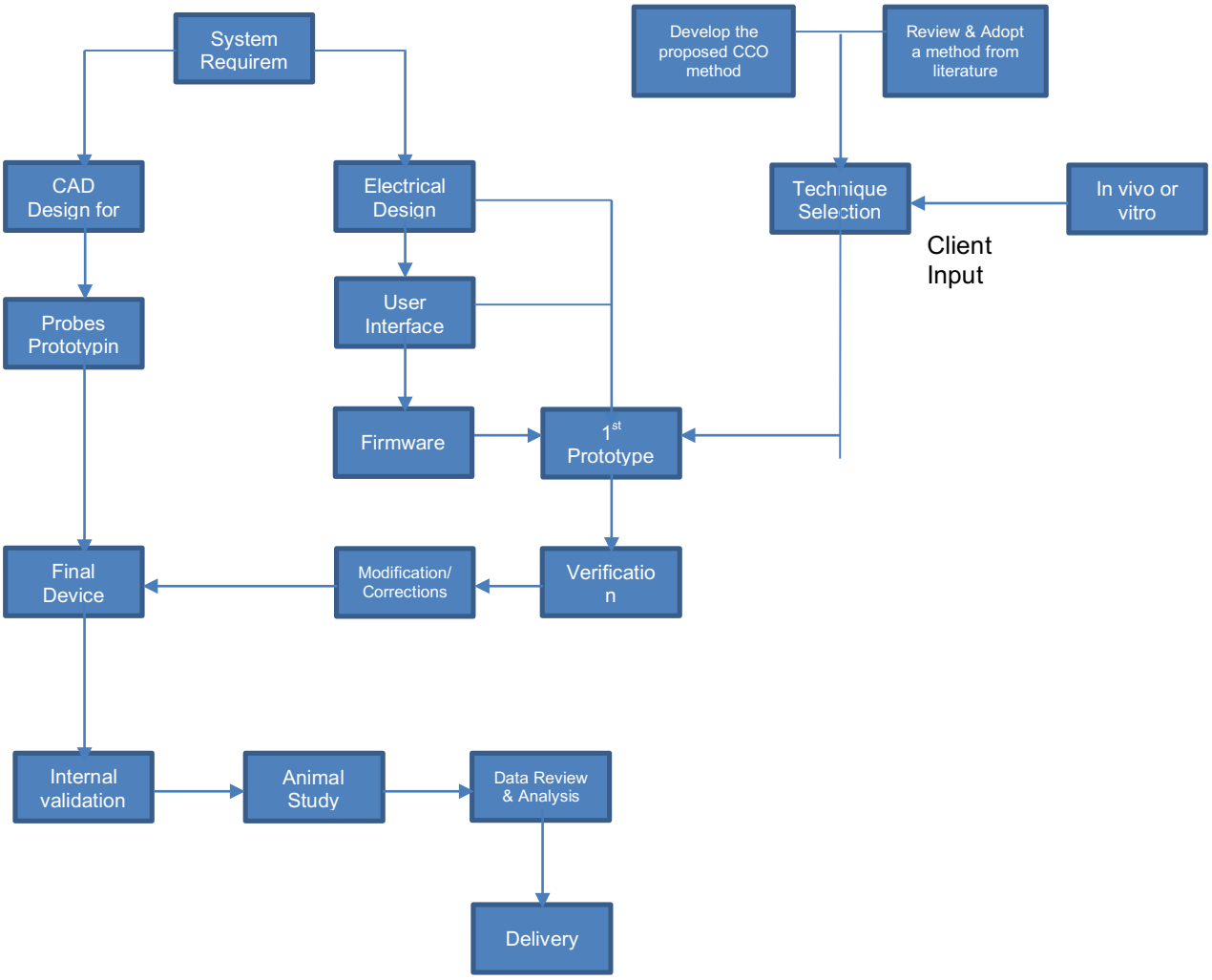
A two channel LED based NIRS device will be developed to measure ΔO_2Hb, ΔHHb, ΔtHb, %TOI and ΔCCO in real time in living tissue. Pathonix has previously developed several systems for measuring the basic parameters (ΔO_2Hb, ΔHHb, ΔtHb and %TOI). Measurement of CCO however, requires development or adoption of an appropriate technique. All parameters except CCO will be calibrated with human subjects in an occlusion test. If the Client has other preferred calibration techniques, they will be adopted for this purpose. Pathonix and the Client agree that CCO measurement may not necessarily result in valid data, however, the effort will be made to get the best possible results.
As this device will be used on exposed spine tissue, the device will be optimized for minimum emitting power and maximum sensitivity.

Pathonix has identified the following specifications for the custom device:

- Continuous wave LED based device
- 5 wavelengths in the range of 650 to 950nm. The exact wavelengths are to be determined, but the most likely options are 670, 770, 810, 850 and 950 nm.
- Penetration depth of approximately 0.6 cm.
- Minimum possible emission power
- Each probe tip will measure less than 2cm by 1.5cm and will house the multi-wavelength LED and the photodectector.
- The probes will be connected to a control unit through flexible wires.
- The control unit connects to a PC or tablet through either a USB cable or Bluetooth.
- A custom Matlab based user interface will be provided to operate the system and record measurements. The measurements will be available in .CSV format for further analysis.
- The device provides the following measures: ΔO_2Hb, ΔHHb, ΔtHb, %TOI and ΔCCO in real time.
- The best effort will be made to make high quality packaging and finishing, but it should be noted that this is a custom-made prototype for research purposes and verification only and may not be in par with requirements for a commercial product.
- The CCO calibration will be based on a method identified and described by the Client.

Project Plan

The following diagram describes the project plan with the major activities and milestones.



The project will begin by the approval of the system requirements outlined in this document along with the project plan by the Client. A monthly update meeting will be held with the Client or their designated representative. At the end of the project, a delivery meeting will be held to approve the deliverables and close the project.

TIMELINE

The following table outlines the time frame for completing the project. The project timeline will initiate upon written confirmation of the client. This timeline serves only as an estimate of the tasks as described and does not account events outside the control of Pathonix. These may include: scope changes, shipment delay, receipt of damaged components, delay in client input.

	Task Description	Week											
		1	2	3	4	5	6	7	8	9	10	11	12
1	System design	X	X	X									
2	CCO Measurement method development & validation		X	X	X								
3	Components sourcing			X									
4	Lead time for components				X	X							
5	1st prototype development						X						
6	Verification/Corrections							X					
7	Enclosure/probe design and production				X	X	X	X					
8	Software development			X	X	X	X	X					
9	Final system (hardware/software), test & refinement								X	X			
10	Internal validation									X			
11	Animal Study										X		
12	Data review and analysis											X	X
13	System delivery												X

Deliverables

The project will be concluded upon delivery of the following.

- *CCO Measurement technique* - A report of the measurement method along with validation process will be provided.
- *Testing data* - The analysis of the test data collected in the animal study will be provided along with conclusion on the validity/reliability of the results and recommendations for further steps.
- *Software* - Custom made MATLAB user interface. The software will be provided as a standalone executable as well as a package containing the source code. The code will be sufficiently commented to allow further development/modifications by the Client.
- *Sensor Hardware* - 2 prototype sensors will be delivered. Each device includes a controller unit and a probe.
- *System Specifications* - Detailed optical and electrical specifications will be provided for future use in the study.